SOME ASPECTS OF OSMOREGULATION IN TWO SPECIES OF SPHAEROMID ISOPOD CRUSTACEA

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The internal effects of osmoregulation were studied in two euryhaline species of isopod crustaceans, *Gnorimophaeroma oregonensis* (Dana) and *Sphaeroma pentodon* Richardson. Although a large literature exists on the subject of osmoregulation in Crustacea, only a little of it concerns isopods. Therefore, the present study was undertaken to gain more information in this relatively unexplored area.

Bogucki (1932) studied the ionic composition of the body fluid in *Mesidotea entomon,* which according to Ekman (1953) is an inhabitant of the Baltic and Arctic Seas and several fresh-water lakes in the land area of the northwest Pacific, Siberia, and northern Europe. Bogucki found the body fluid concentration to be hypertonic to the medium in lower salinities, becoming isotonic as the medium approached sea water. Lockwood and Croghan (1957), studying the brackish- and fresh-water races of the same species, found the osmotic behavior to be similar in both races, except that the brackish-water animals could not survive in fresh water. They concluded that the fresh-water race has developed a more effective osmoregulatory mechanism that enables it to maintain the high haemolymph concentrations of the brackish-water race in fresh water. Bateman (1933) found that *Ligia oceanica* maintained its body fluid hypertonic to a medium of about 80 per cent sea water, but swelled and died in 50 per cent sea water. However, Parry (1953), working with *Ligia exotica,* found that in well-aerated sea water, specimens of the species could survive 17 to 30 days in salinities ranging from 50 to 125 per cent sea water. In very concentrated media ($\Delta = 3.46°$ C.), the body fluid was maintained hypotonic to the medium. Menzies (1954), in addition to splitting *Gnorimosphaeroma oregonensis* into two subspecies, *lutea* and *oregonensis,* performed preliminary experiments to test the ability of the two subspecies to survive in various salinities. Specimens of *G. o. oregonensis* taken from 25 per cent sea water and placed in tap water were all dead after one day. Specimens of *G. o. lutea* taken from 1.6 per cent sea water and placed in tap water died slowly over a period of three days. Menzies concluded that *G. o. oregonensis* is probably restricted to sea water, but he was puzzled as to why *G. o. lutea* could survive in sea water, but not in tap water (salts equivalent to 0.3% sea water), which was not very much less saline than the normal habitat water (1.6% sea water). He postulated that *G. o. lutea* required a slight concentration of salts, greater than the tap water used, or that there were toxins present in that medium.

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MATERIALS AND METHODS

Experimental animals

Gnorimosphaeroma oregonensis is widely distributed over the west coast of North America from Alaska to central California (Menzies, 1954). It also occurs in Hawaii (M. A. Miller, unpublished report). It may be collected intertidally in bays, in estuarine conditions and occasionally populations of the species are found in fresh-water creeks and ponds. Because of its ability to inhabit such a wide ecological range, it was considered to be a suitable experimental type for the further elucidation of osmoregulatory adaptations which enable a marine animal to live in brackish and fresh water.

The following designations will be used for the three habitat groups of *Gnorimosphaeroma oregonensis*. Animals taken from fresh water will be called *G. oregonensis* (FW); those taken from estuarine populations will be called *G. oregonensis* (EF = estuarine form), and those animals taken from intertidal bay populations will be designated *G. oregonensis* (BF = bay form). *G. oregonensis* (FW and EF) equal the subspecies *G. o. lutea* of Menzies. *G. oregonensis* (BF) equals the subspecies *G. o. oregonensis* of Menzies.

Sphaeroma pentodon is known only from San Francisco Bay (Richardson, 1905), Tomales Bay (new locality), and Bolinas Lagoon (new locality), California. It lives intertidally in burrows, which it constructs in mud, wooden logs and pilings, and sandstone. The salinity of the habitats from which it was collected ranged from about 11 per cent sea water to about 96 per cent sea water. *S. pentodon* was included in the study because it is related to *Gnorimosphaeroma oregonensis*, and its range overlaps that of the latter species in parts of its distribution.

Methods

Four principal types of studies were made: (1) Changes in the total osmotic pressure of the body fluid after three, 24, and 48 hours exposure to the experimental salinities were made in order to determine the relative degree and pattern of osmoregulation exhibited by the animals. (2) The animals were weighed before and after exposure to the experimental salinities for 24 hours in order to detect possible changes in weight indicating water gain or loss. (3) Survival tests were run to determine the length of time the experimental animals could live in the experimental salinities. (4) Field checks were made by measuring changes in the body fluid of all but *Gnorimosphaeroma oregonensis* (FW) during a portion of a tidal cycle.

The laboratory experiments were conducted at 16° C., a temperature to which all forms were accustomed. The animals were placed in 60 per cent sea water for 24 hours prior to the start of the experiments. The 60 per cent sea water permitted a common starting salinity for all experimental series, facilitating comparisons.

Experimental salinities

The experimental salinities used in this study were 125, 100, 75, 50, and 25 per cent sea water, and fresh water (salts equivalent to 0.25% sea water by chloride determination). The 100 per cent sea water (salinity = 34.44‰) was collected off the Marin County coast away from the influence of fresh-water streams. The 25, 50, and 75 per cent sea water solutions were made by diluting normal sea water with distilled water. The 125 per cent sea water solution was prepared by boiling normal sea water, taking care not to precipitate salts. The pH was checked before and after boiling to ascertain that any loss of carbon dioxide was regained by exposure to air. The fresh water was soft creek water collected at Pilarcitos Creek, San Mateo County, California.

Salinity determinations

Salinity determinations on sea water concentrations greater than 25 per cent sea water were made by a short method described by Welsh and Smith (1953). The salinity of sea water diluted to less than 25 per cent normal sea water and fresh water was determined by the standard silver nitrate titration method using the Knudsen Tables (1901).

Melting point determinations

A method devised by Gross (1954) was used for determining the melting point of body fluids. From repeated runs on standard samples, it was found that the concentration of the body fluids could be obtained within an error of about two per cent sea water (0.04° C.).

Body fluid samples (*ca.* 1–2 mm^3.) were collected into prepared melting point capillaries (*ca.* 1 mm. ID × 3 cm. length) which were previously marked with a coded series of dots corresponding to the experimental salinities to which the animals had been exposed. Collection of the body fluid was facilitated by the use of a hand control. After collection, both ends of the capillary were sealed with petroleum jelly and the sample quick-frozen on dry ice.

Survival tests

The ability of the experimental animals to survive for extended periods of time in the experimental salinities was tested as follows: Seventy animals of each experimental group were placed, ten each, in six jars containing the experimental salinities, and one jar containing filtered habitat water. The jars were checked daily for 21 days, and the number of survivors recorded.

Field tests

Changes in the body fluid concentration of *Gnorimosphaeroma oregonensis* (EF), *G. oregonensis* (BF), and *Sphaeroma pentodon* during a 7½-hour period from low to high tide in the field were measured as follows: In the case of *G. oregonensis* (EF), which remains immersed in water during low tide, five body fluid samples and one sea water sample were taken at 1¼-hour intervals. In the case of *G. oregonensis* (BF) and *S. pentodon,* which remain out of the water during low tide, five body fluid samples and five samples of water around the pleopods were collected. The body fluid and pleopod water samples were frozen on dry ice and returned to Davis for determination.

RESULTS

The term "gradient" will be used in the following pages to indicate the difference in concentration (expressed in percentage sea water) between the body fluid and the medium.

Melting point determination of body fluid concentrations

The results of melting point determination of body fluid concentrations are shown in Figure 1. In general, changes in the body fluid concentrations seemed to be rapid—the major changes occurred within the first three hours of exposure to the experimental salinities.

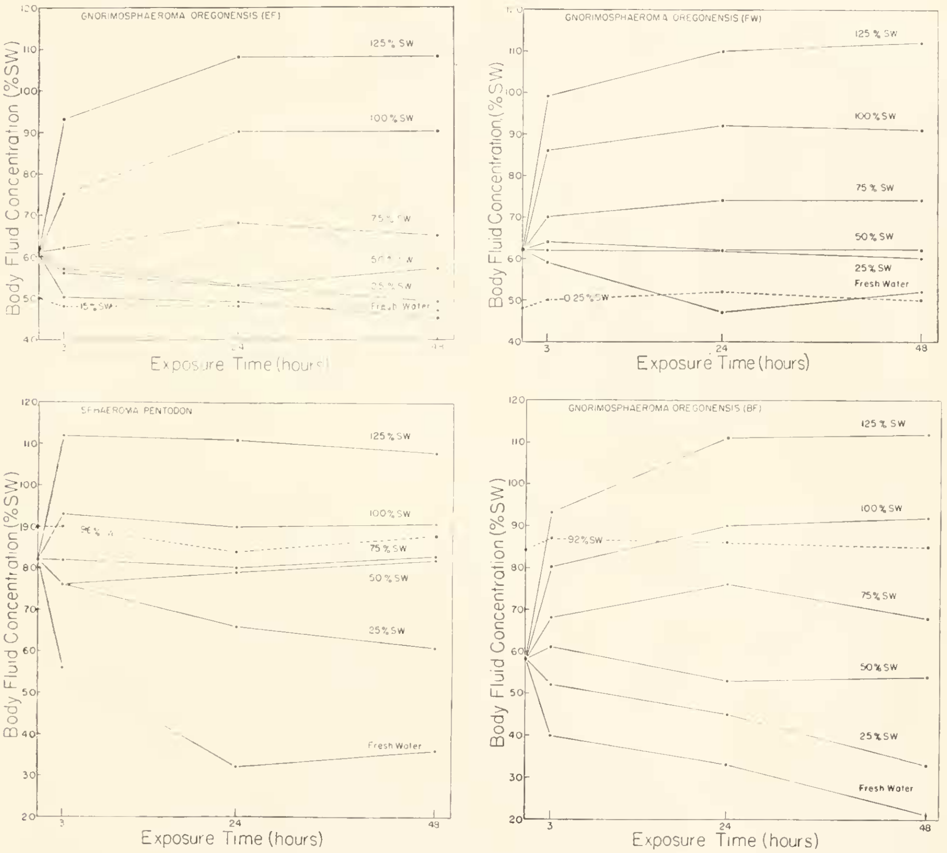

FIGURE 1. Body fluid concentration changes with time in the experimental salinities. The dotted line represents the body fluid concentration changes of animals kept in habitat salinities (controls) indicated.

After 48 hours' exposure, the fresh-water and estuarine forms of *Gnorimosphaeroma oregonensis* maintained their body fluids hypertonic to the medium in 50 per cent sea water and less, and hypotonic in 75 per cent sea water and above. However, in 75 per cent sea water after 24 hours' exposure, the body fluid concentration values of *G. oregonensis* (FW) were quite variable, ranging between hypotonicity and hypertonicity. Possibly that salinity is close to the medium concentration where the "switch" from hyper- to hypo-osmotic regulation occurs. *G. oregonensis* (BF) maintained its body fluid hypotonic to the medium in 75 per cent sea water and above, and hypertonic to 50 and 25 per cent sea water. Apparently, there was no maintenance of the body fluid concentration in fresh water. In that medium, the body fluid concentration steadily dropped, and after 48 hours, all of the animals were dead.

Comparing the above results with those of Menzies (1954) above it can be seen that in both studies, *Gnorimosphaeroma oregonensis* (BF) (= *G. o. oregonensis* of Menzies) could not survive in fresh water. However, in Menzies' study, *G. oregonensis* (EF) (= *G. o. lutea* of Menzies) were not surviving after three days in tap water, while in the present study, that form lived for several days in fresh water. It is possible that the tap water used by Menzies (unchlorinated well water) contained some unknown toxic substance or had an imbalance of ions. Its ion analysis is as follows: HCO_3, 0.241‰; SO_4, 0.037‰; Cl, 0.029‰; Ca, 0.011‰; Mg, 0.020‰; and Na, 0.078‰.

After 48 hours, *Sphaeroma pentodon* maintained its body fluid hypotonic to the medium in 100 and 125 per cent sea water and hypertonic in the lower salinities. It is interesting to note that *S. pentodon* and *Gnorimosphaeroma oregonensis* (BF) have extremely wide viability limits in terms of the concentration and dilution of their body fluids—surviving within a concentration range (of their body fluids) of over 70 per cent sea water!

Weight changes in the experimental media

No weight changes were detected in any of the experimental animal groups, except *Gnorimosphaeroma oregonensis* (BF) in fresh water. In that salinity, the majority of the animals were very close to death at the end of the 24-hour period, and the weight changes were considered to be subnormal. Those animals that were still active at the end of the 24 hours did not lose weight. It was possible to weigh the animals within an average error of one per cent of their body weight.

Survival tests

The survival experiment was terminated after 21 days. At termination, the estuarine and fresh-water forms of *Gnorimosphaeroma oregonensis* were surviving in all salinities. *G. oregonensis* (BF) was surviving in all salinities except fresh water, where the LD_{50} value (average survival time) was less than two days. *Sphaeroma pentodon* was surviving in all experimental salinities, except fresh water, where the LD_{50} value was 11 days. No unusual mortality was noted among the controls.

Field tests

The results of the field test of body fluid concentration changes during a tidal cycle showed that no significant changes in concentration of the body fluid or water surrounding the pleopods were detected in *Gnorimosphaeroma oregonensis* (BF) or *Sphaeroma pentodon*. In *G. oregonensis* (EF), however, changes were rather characteristic. Starting at low tide, when the animals were exposed to fresh water, the body fluid concentration was 50 per cent sea water. This concentration did not change until over five hours later, when the salinity of the habitat had reached 42 per cent sea water, at which time the body fluid concentration was 58 per cent sea water. Then, by the time of the extreme high tide, 1¼ hours later, the body fluid concentration had changed again to 70 per cent sea water, while the medium concentration had changed to 65 per cent sea water.

Discussion

Comparative osmoregulatory abilities

Figure 2 shows the 48-hour body fluid concentrations of the experimental animals in the experimental media. It was assumed that all major changes in body fluid concentration had occurred by 48 hours. In hypotonic media, *Sphaeroma pentodon* appears to be a strong regulator, at least in 50 and 75 per cent sea water. There is no apparent reason for the animals to maintain such high body fluid concentration in those salinities when they can live, at least for several days, in fresh water and 25 per cent sea water with (presumably) much lower body fluid concentrations. *Gnorimosphaeroma oregonensis* (BF) has only limited regulation in all media and appears to be the greatest conformer of the group, maintaining a relatively small gradient between its body fluid and the medium in all salinities. *G. oregonensis* (EF) and *G. oregonensis* (FW) are the most able regulators in terms of the ability to maintain their body fluid concentrations relatively constant in hypotonic media. The body fluid concentration differences between the two forms seen in fresh water, 25 per cent sea water, and 50 per cent sea water, are statistically significant ($t = 6.15$, 3.87, and 12.3, respectively, with 11, 10, and 9 degrees of freedom). The ability of *G. oregonensis* (FW) to maintain its body fluid more concentrated in the hypotonic media perhaps represents the major osmoregulatory difference between the two forms. The estuarine form is intermediate between the bay and fresh-water forms in osmoregulatory ability.

Comparing the osmoregulatory abilities of the isopods in this study with those of other crustaceans, a similarity can be seen to species inhabiting similar salinity ranges. From the results of Lockwood and Croghan (1957), it appears that *Mesidotea entomon* is similar in its osmotic regulation to *Gnorimosphaeroma oregonensis*. The former species consists of two "races" which have adapted to brackish- and fresh-water. As in *G. oregonensis* (FW), the fresh-water *M. entomon* is able to live in salinities up to normal sea water. However, unlike *G. oregonensis* (EF), the brackish-water "race" of *M. entomon* cannot live in fresh water. The brackish-water *M. entomon* is thus closer to *G. oregonensis* (BF) and *Sphaeroma pentodon* in its osmoregulatory abilities. However, *M. entomon* does not show the high degree of hypo-osmotic regulation seen in the isopods in the present study. Beadle and Cragg (1940) reported a difference in the ability to retain chloride between the brackish- and fresh-water forms of the amphipod, *Gammarus duebeni*, when placed in distilled water. The fresh-water form retained sufficient chloride to survive for several days in distilled water, whereas the brackish-

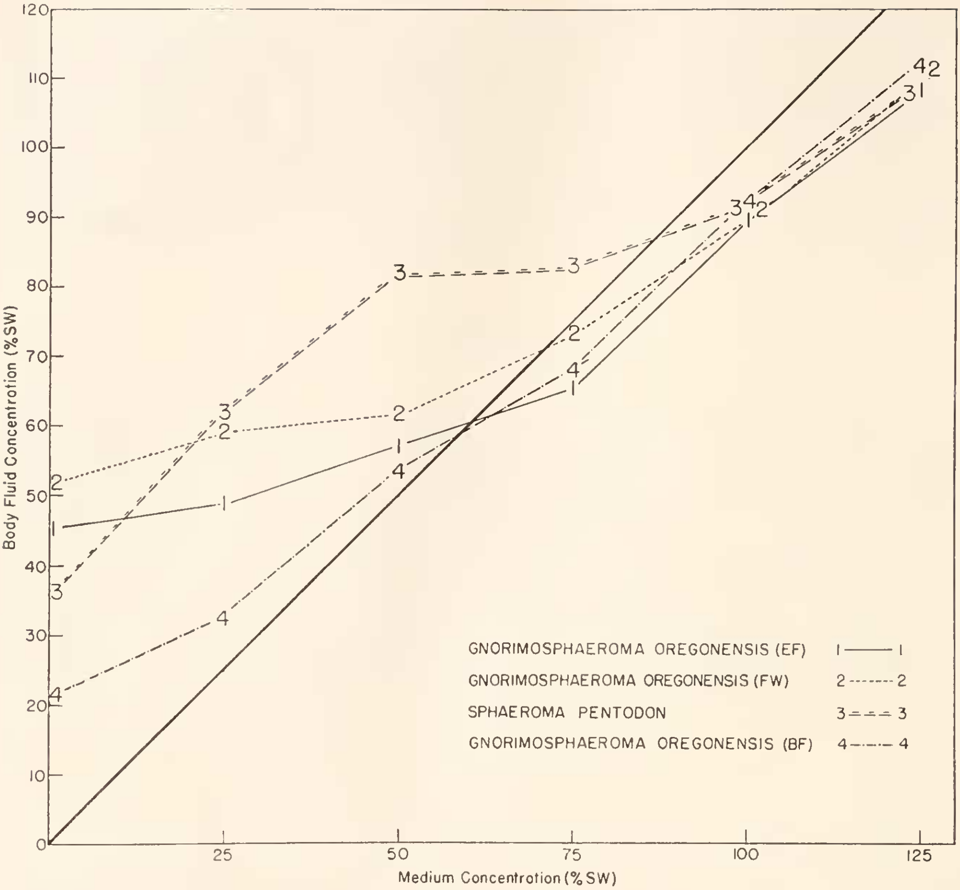

FIGURE 2. Relation of the body fluid concentration to the medium concentration of animals exposed for 48 hours to the experimental salinities.

water form lost chloride and died rapidly in that medium. It appears that the osmoregulatory abilities of the isopods in this study are intermediate between those of one group of crustaceans which can hyper-regulate in dilute sea water, but become isosmotic, or nearly so, in salinities approaching normal sea water (*e.g., Carcinus maenas,* Schlieper, 1929; *Hemigrapsus oregonensis* and *H. nudus,* Jones, 1941) and a second group of crustaceans, which hyper-regulate in dilute sea water and hypo-regulate in salinities approaching normal sea water (*e.g., Heloecius cordiformis,* Edmonds, 1935; *Uca crenulata* and *Pachygrapsus crassipes,* Jones, 1941; *Palaemonetes varians,* Panikkar, 1941; and *Palaemon serratus,* Parry, 1954). All but the last two members of the latter group are primarily semi-terrestrial, which has led Prosser *et al.* (1950a, 1955) to suggest that hypo-osmotic regulation may be associated with the semi-terrestrial habit. The isopods in this study are able to survive for extended periods out of water, but they cannot be classified as semi-terrestrial.

The mechanism of osmoregulation

Although there is little direct evidence elucidating the actual mechanisms of osmotic regulation of the body fluid of the experimental animals, it is possible to make certain hypotheses concerning that phenomenon based on data obtained in the present study and in studies (unpublished) which were made prior to the present study.

a. *Evidence for water movement*

There were no detectable weight changes in the experiments conducted at 16° C., which indicates that there was no net gain or loss of water from the experimental animals' bodies. It is probable that the maintenance of a zero net water flux (that is, no imbalance of the water gain/loss ratio) is dependent upon the ability of the animal to maintain its metabolic rate at a normal level. Duplicate experiments done at 5° C. (see Riegel, 1958) resulted in weight gains by the experimental animals in the dilute salinities and weight losses in the more concentrated salinities. These results may be interpreted as being due to an interference by the low temperature with the normal metabolism of the animals.

b. *Evidence for salt movement*

Since there were no weight changes in the experiments conducted at 16° C., it must be assumed that body fluid concentration changes were due to salt movement. In dilute media (fresh water to 50 per cent sea water), the salt concentration of the body fluid was actively maintained against a gradient. In more concentrated media (75 to 125 per cent sea water), salts were prevented from entering the body (or were eliminated as fast as they came in), since after the initial concentration of the body fluids (generally by 24 hours) the body fluid was maintained hypotonic to the medium. This mechanism could possibly involve, at least in part, an arrest of the mechanism for active salt absorption.

Except for *Gnorimosphaeroma oregonensis* (FW) there was a rapid loss of salts (within three hours) in the more dilute salinities. Whether this loss was due to an active elimination of salts by the animal, thus reducing the concentration gradient between their body fluids and the medium, or a passive loss from the body is not known. There is some evidence suggesting an active elimination of salts in the more dilute salinities, shown especially by *G. oregonensis* (EF) and *Sphaeroma pentodon* after three hours' exposure to fresh water. In those two forms, the body fluid concentrations dropped more rapidly at 16° C. than at 5° C. (see Riegel, 1958).

Whatever the mechanism for the maintenance of the body fluid concentrations in lower salinities, low temperatures interfere with the metabolism of the animals, causing variations in osmoregulation not seen at the higher temperature. In all cases, except *Gnorimosphaeroma oregonensis* (BF) in fresh water, the animals were able to maintain their body fluid concentrations within viable limits after 48 hours' exposure at 16° C. But at 5° C., *G. oregonensis* (BF) was dead after 24 hours' exposure to fresh water and 48 hours' exposure to 25 per cent sea water, and *Sphaeroma pentodon* died after 48 hours' exposure to fresh water. Further, the body fluid concentration of *G. oregonensis* (FW) and *G. oregonensis* (EF) dropped to subnormal values in fresh water at the lower temperature, but remained within normal limits at the higher temperature.

Wikgren (1953) studied the effect of low temperature on various poikilothermous animals (a crayfish, a lamprey, and a bony fish) and concluded that low temperatures have their chief effect in interfering with the ion-absorbing mechanism of the animals. In the lamprey, urine production was decreased by low temperature, which may have resulted in a weight gain, although Wikgren did not indicate that such was the case. David (1925) performed experiments on the living kidney of the frog, which indicated that that organ's urine diluting and concentrating activity was not affected by temperature. However, Wikgren (1953) recalculated David's data and stated that the diluting capacity of the frog's kidney was reduced by low temperature. Thus, evidence may be inferred from the review by Wikgren (1953) that low temperature adversely affects the ability of cold-blooded animals (at least, cold-blooded vertebrates) to rid the body of water.

The changes in body fluid concentration seen in the present study at 16° C. were undoubtedly due to salt movement. Since there were demonstrated water losses and gains at 5° C., the question arose as to whether the body fluid concentration changes which occurred at that low temperature were due entirely to water movement or were partly due to salt movement.

Because the usual procedures for determining body fluid volume were hardly applicable to animals of such small size as used in this study, that component was estimated in the following manner. Ten animals of each experimental group were weighed, and all the body fluid removed from their bodies that could be collected into capillaries of 1-mm. bore. The animals were then re-weighed. Average collectable body fluid weights as a percentage of total body weight were 9.5, 9.7, 11.1, and 6. 8, respectively, for *Gnorimosphaeroma oregonensis* (FW), *G. oregonensis* (EF), *G. oregonensis* (BF), and *Sphaeroma pentodon.* These values established the minimum possible weight of the body fluid. Ten animals of each experimental group were weighed and dried to constant weight in a calcium chloride desiccator. The average values for total body water as a percentage of the total body weight were 56.5, 55.6, 56.4, and 53.8, respectively, for *G. oregonensis* (FW), *G. oregonensis* (EF), *G. oregonensis* (BF), and *S. pentodon.* These values established the maximum possible weight of the body fluid as a percentage of the total body weight.

Table I compares the calculated and actual dilution and concentration of the body fluids in fresh water and 125 per cent sea water [using a 40-milligram specimen of *Gnorimosphaeroma oregonensis* (EF) as an example] based on estimates of the body fluid weight ranging from ten to 50 per cent of the total body weight. A sample calculation follows: Referring to Table I, it can be seen that a 40-milligram animal, with a body fluid concentration of 50 per cent sea water (column 5), when placed in fresh water would gain 11.3 per cent of its body weight (column 3) after 24 hours. If the weight gain is due entirely to water entry into the body, the incoming water would dilute the body fluids by a factor, X, given by the relation: $X = \frac{\text{wt}_0\ (= \text{original body fluid weight})}{\text{wt}_{24}\ (= \text{24-hour body fluid weight})}$. If the body fluid comprises 50 per cent of the total body weight (column 1), its dilution by the gain of 4.5 milligrams of water (column 4) would result in a body fluid concentration of $X\cdot 50$ $\left(=\frac{20}{24.5}\cdot 50\right)$, or 40.8 per cent sea water (column 6).

When a 40-milligram animal whose initial body fluid concentration is 50 per cent sea water is placed in 125 per cent sea water, if the body fluid comprises 50 per cent of the total weight, the body fluid would be concentrated by the factor $X\left(=\frac{20}{17.3}\right)$. Thus the body fluid will be concentrated to 57.8 per cent sea water (column 10).

TABLE I

Comparison of actual and calculated concentration and dilution of the body fluids (BF) at 5° C. based on several estimates of the body fluid weight (BF Wt.) as a percentage of total body weight (BW) and assuming the concentration and dilution to be due entirely to water movement*

1 Est. BF Wt. (% BW)	2 Est. BF Wt. (mg.)	3 % BW gain FW	4 BF Wt. after 24 hrs. FW	5 Start. BF conc. (% SW)	6 Calc. BF conc. FW	7 Actual BF conc. 24 hrs. FW	8 % BW loss 125% SW	9 BF Wt. 24 hrs. 125% SW	10 Calc. BF conc. 24 hrs. in 125% SW	11 Actual BF conc. 24 hrs. in 125% SW
50	20	11.3	24.5	50	40.8	42	6.7	17.3	57.8	112
40	16	11.3	20.5	50	39.0	42	6.7	13.3	60.2	112
30	12	11.3	16.5	50	36.4	42	6.7	9.3	64.5	112
20	8	11.3	12.5	50	32.0	42	6.7	5.3	75.4	112
18	7.2	11.3	11.7	50	30.8	42	6.7	4.5	80.0	112
16	6.4	11.3	10.9	50	29.4	42	6.7	3.7	86.5	112
14	5.6	11.3	10.1	50	27.7	42	6.7	2.9	96.6	112
13	5.2	11.3	9.7	50	26.8	42	6.7	2.5	104.0	112
12	4.8	11.3	9.3	50	25.8	42	6.7	2.1	114.3	112
11	4.4	11.3	8.9	50	24.7	42	6.7	1.7	129.4	112
10	4.0	11.3	8.5	50	23.5	42	6.7	1.3	153.8	112

* A 40-mg. specimen of *Gnorimosphaeroma oregonensis* (EF) was used as an example.

From Table I it can be seen that the calculated body fluid concentrations and dilutions in 125 per cent sea water and fresh water, based on estimates of the body fluid weight percentage, do not completely match the actual results. If the estimated body fluid weight of 50 per cent total weight is correct, the calculated dilution in fresh water is close to the actual value. However, the calculated concentration in 125 per cent sea water is much lower than the actual value. If the estimated body fluid weight of 12 per cent total body weight is correct, the calculated body fluid concentration in 125 per cent sea water is close to the actual value, but the calculated body fluid concentration in fresh water is much lower than the actual value. Therefore, it is likely that the actual body fluid weight lies somewhere between 10 and 50 per cent of the total body weight. If a reasonable estimate of 20 to 30 per cent is close to the actual value for the body fluid component of the total body weight, it appears that the actual body fluid concentrations in fresh water and 125 per cent sea water at 5° C. are not due entirely to water movement. That is, it is probable that there is a retention or reabsorption of salts in fresh water and an absorption of salts in 125 per cent sea water.

These results are in general agreement with those of Hukuda (1932) who compared the theoretical and actual change in weight with the observed change in osmotic pressure of the blood in *Portunas puber* when that marine animal was immersed in ⅔ normal sea water. Gross (1957) found in *Emerita analoga* that a weight change of less than two per cent of the body weight resulted in a body fluid concentration change equivalent to 25 per cent sea water. Based on the assumption that osmotically active water comprised 40 per cent of the body weight, he calculated that the weight change, if due entirely to water movement, would have changed the body fluid concentration by less than six per cent.

The estimate of 20 to 30 per cent as the haemolymph component of the body weight in *Gnorimosphaeroma oregonensis* (EF) only partially agrees with similar estimates of that value in other crustaceans. A body fluid value of 50 per cent of body weight was assumed by Lockwood and Croghan (1957) for *Mesidotea entomon*. Similarly, a body fluid of ⅓ body weight was assumed for *Palaemonetes antennarius* by Parry (1957). Gross (1957) made actual calculations of the "solute space" in *Pachygrapsus crassipes* and *Emerita analoga* which were, respectively, 56 and 40 per cent of body weight. However, solute space would be expected to be greater than the body fluid volume and less than the total body water. Approximate measurements of blood volume of various crustaceans have been made using sodium thiocyanate. Nagel (1934) found a blood volume of 37 per cent of body weight in *Carcinus maenas*. Krogh (1939) measured a blood volume of 33 per cent of body weight in *Eriocheir sinensis*. Prosser and Weinstein (1950) measured the body fluid volume of the crayfish, *Orconectes virilis,* obtaining values of 25.6 per cent and 25.1 per cent, respectively, with sodium ferrocyanide and a dye, T-1824. The isopods in the present study seemed to have large amount of exoskeleton relative to soft tissue. This fact was further borne out by the relatively low total water values, and in the writer's opinion, supports the estimate of 20 to 30 per cent of total body weight as the body fluid component.

To summarize, it is probable that the osmoregulatory abilities of the experimental animals include a mechanism for active salt uptake and retention. In the experiments conducted at 16° C., the body fluid concentrations and dilutions were not accompanied by detectable weight losses or gains, suggesting that the concentration and dilution are due to salt movement. Since concentrations and dilutions of the body fluids could not be explained purely on the basis of water movement (weight losses or gains), in experiments conducted at 5° C., there is evidence that concentration changes, especially in the higher salinities (75 to 125 per cent sea water) were also due to salt movement at the low temperature. There is some evidence that the experimental animals actively maintain the normal water content of the body fluid. Though body fluid concentrations were well-marked at 16° C., no weight changes were detected. Rather than propose that no water enters or leaves the bodies of the experimental animals upon exposure to the experimental salinities, it might be more reasonable to assume that the normal body water component is actively maintained by pumping water out as fast as it comes in in hypotonic media and by active water uptake and/or salt elimination in hypertonic media. The fact that weight changes were well-marked in experiments conducted at 5° C. and non-existent in experiments conducted at 16° C. indicates that the mechanism for active maintenance of the water balance of the body is depressed or inactivated by low temperature.

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SUMMARY

1. Osmoregulatory requirements were analyzed and compared in Menzies' two subspecies of *Gnorimosphaeroma oregonensis* (*G. o. oregonensis* and *G. o. lutea*) and *Sphaeroma pentodon* Richardson.

2. The mechanism of osmoregulation was studied by measuring changes in the total osmotic concentration of the body fluid after three to 48 hours' exposure to various experimental salinities ranging from fresh water to 125 per cent sea water. Changes were also measured in the field during a partial tidal cycle. The principal findings and conclusions are as follows:

a.) The body fluids of the experimental animals became either diluted or concentrated in the experimental salinities. Generally, in more dilute media (50% sea water or less), the body fluids were maintained hypertonic to the medium, while in more concentrated media (75 to 125% sea water), they were usually maintained hypotonic to the medium.

b.) The lack of weight changes in experimental salinities in experiments conducted at 16° C. indicates that dilution and concentration of the body fluid at normal temperatures are caused primarily by salt movement.

c.) Pronounced weight changes that occurred in experiments conducted at 5° C. suggest that the normal water component of the body fluid is actively maintained and that low temperature interferes with this active maintenance, which normally permits excess water to leave the body in diluted media and to enter in more concentrated salinities. However, the fact that the degree of concentration and dilution of the body fluids at the low temperature could not be explained solely on the basis of water movement suggests concurrent salt gains or losses.

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